

An aerial photograph of a city, likely Lima, Peru, showing a river winding through the urban landscape. The river is a light brown color, contrasting with the green parks and the grey urban grid. The text is overlaid on the top half of the image.

hypothetical case study: Insect-resistant, herbicide-tolerant maize for unrestricted release in Mexico

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Maize production in Mexico

- **Production scale:**

- Micro:

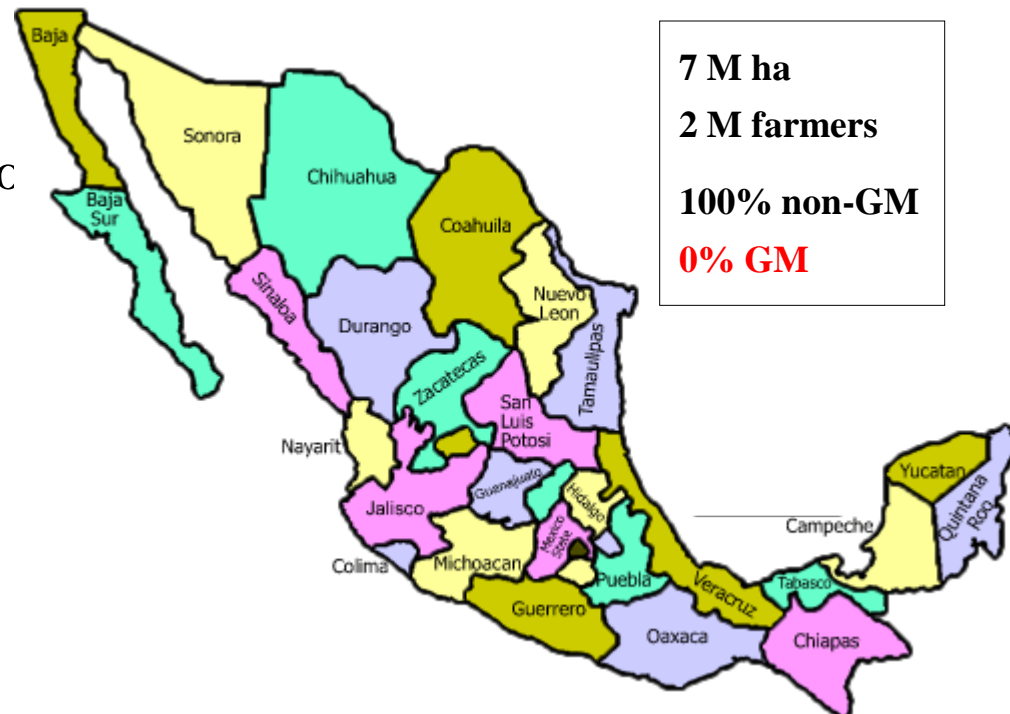
- Landraces for self consumption or local trade

- Small to medium:

- on land < 5 ha
- for trade
- Improved landraces & seed
- Technology often used

- Macro:

- 23% of land > 5 ha
- Improved seed for market demands
- Technology-driven production



Maize consumption worldwide

White and yellow maize are most consumed

Human consumption

- In Africa & Central America: white maize
- in South America: yellow maize

Uses:

- Staple food: maize meal (eg, corn bread, tortillas), maize grain (e.g. pozole).
- Other: corn oil, maize starch, high-fructose syrup, ethanol

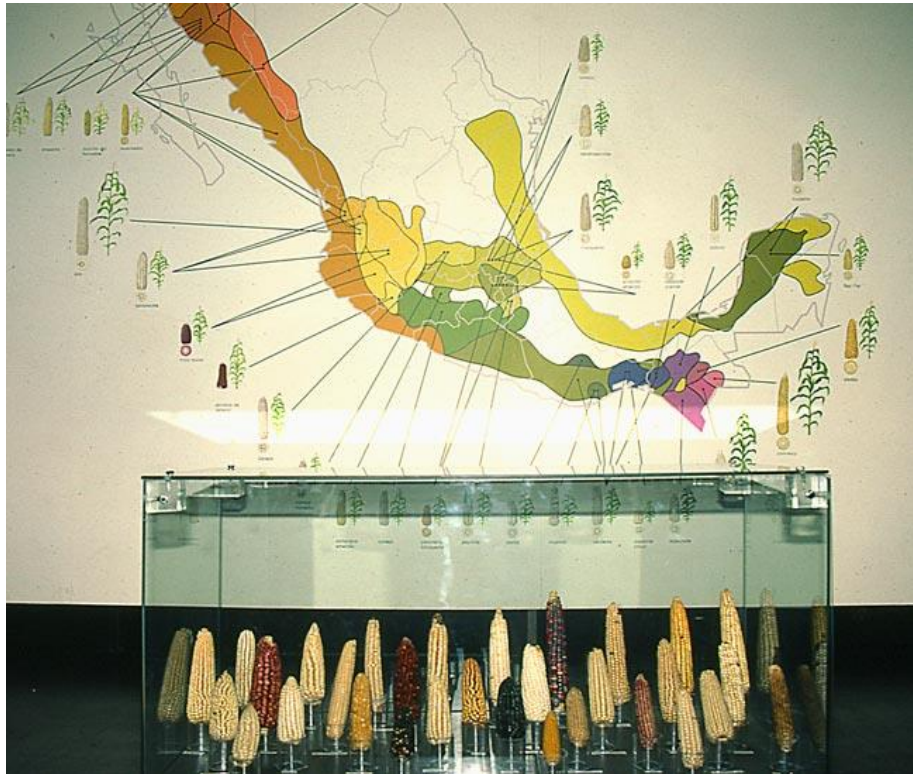
Animal consumption

- Worldwide widespread use of yellow maize



Importance of maize in Mexico

in terms of food, economy & socio-cultural traditions



Exhibition on history of corn
(Museum of Popular Culture, Mexico)



elotes (steamed corn cobs)



Xilonen

(Aztecs' name for young fresh maize)



tortilla chips

Centre of origin

= the geographical area where process of domestication took place (~10,000 years ago)*

- Mexico & Guatemala = centre of origin of maize
- Ancestor: **teosinte** (*Zea mays* spp. *mexicana*; *Zea mays* spp. *parviglumis*)

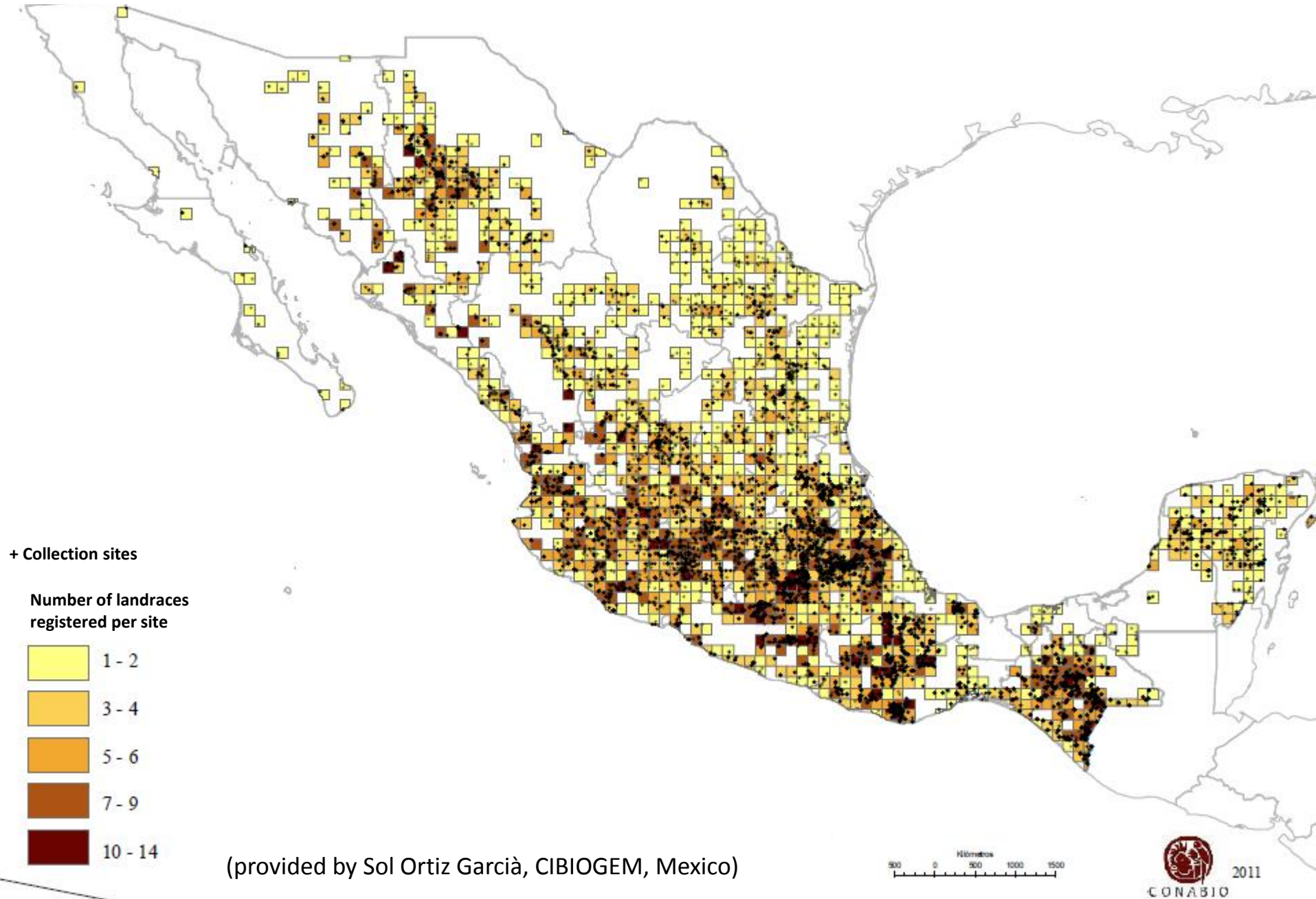
Classification by Iltis & Doebley (1980) and Doebley & Iltis (1980)

- Maize: *Zea mays* spp. *mays*
 - ~ 59 landraces in Mexico
 - cross-compatible with teosinte
($< 1\%$ *Z. mays* spp. *mexicana*; $> 50\%$ *Z. mays* spp. *parviglumis*)

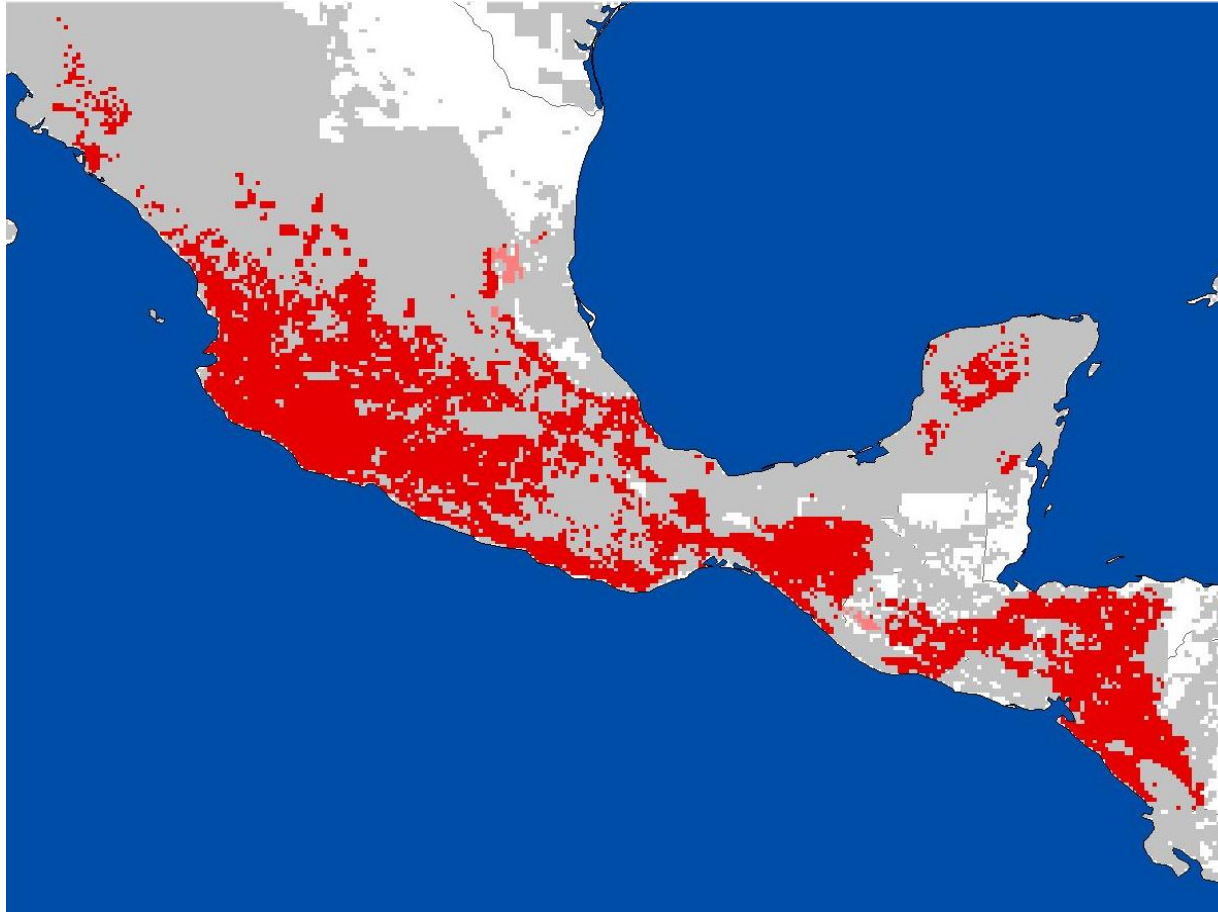


*American Society of Plant Biologists (2008, June 27).

Presence of landraces in Mexico (1940-2010)



Likelihood of maize-teosinte hybridisation

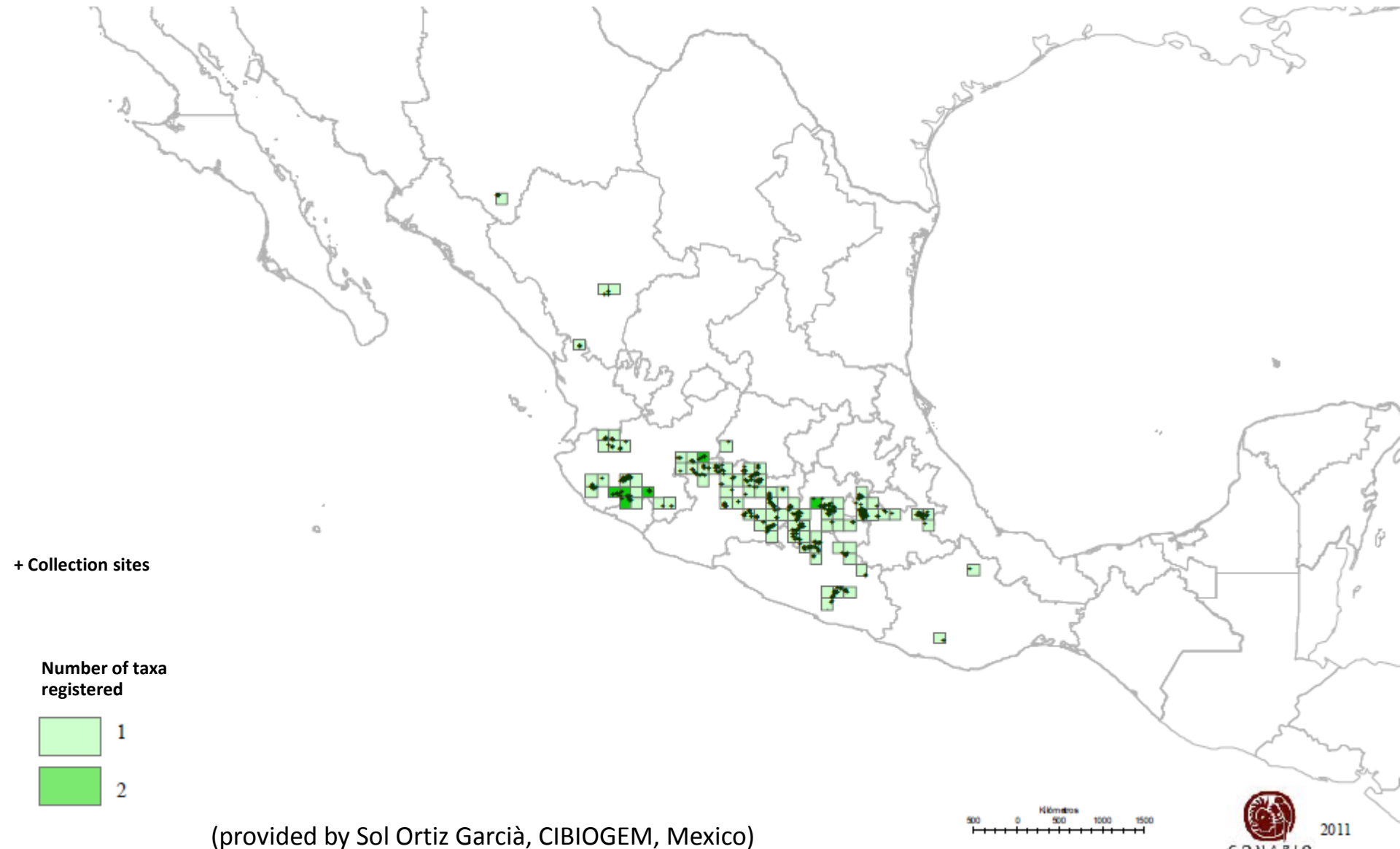


High likelihood of hybridisation of crop with:
Zea diploperennis, *Z. luxurians*, *Z. nicaraguensis*, *Z. mays* subsp.
huetenangensis, *Z. mays* subsp. *Mexicana*, *Z. subsp parviglumis*

Only crop or only crop wild relatives (i.e, no overlap)

Presence of teosinte in Mexico (1940-2010)

(*Z.mays* ssp *parviglumis* & *mexicana*)



Case-study: GM triple-stack maize

- **Host organism:**
- *Zea mays* spp. *mays* L. (maize)
- **Traits:**
- Event 1: resistance to lepidopteran pests (Cry1Ab)
- Event 2: resistance to coleopteran pests (Cry3Bb1)
- Event 3: tolerance to herbicide Round-up (CP4 EPSPS)
- ↓
- Events combined by traditional plant breeding → triple stack

Host organism biology

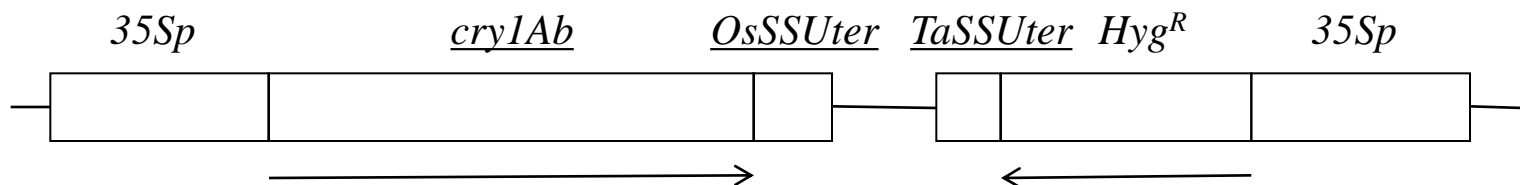
- Maize is predominantly a wind-pollinated, outcrossing species
- Maize is monoecious, with male and female reproductive organs being borne on the same plant as separate inflorescences.
- Pollen is borne on specialised male inflorescences called tassels, whilst ovules are enclosed in ears with stigmas protruding as silks
- Maize is cross-compatible with teosinte
($< 1\%$ *Z. mays* ssp. *mexicana*; $> 50\%$ *Z. mays* ssp. *parviglumis*)
- Maize is not known to become weedy, due to traits such as lack of seed dormancy & poor competitiveness of seedlings.



Event 1: Cry1Ab

Trait and modification

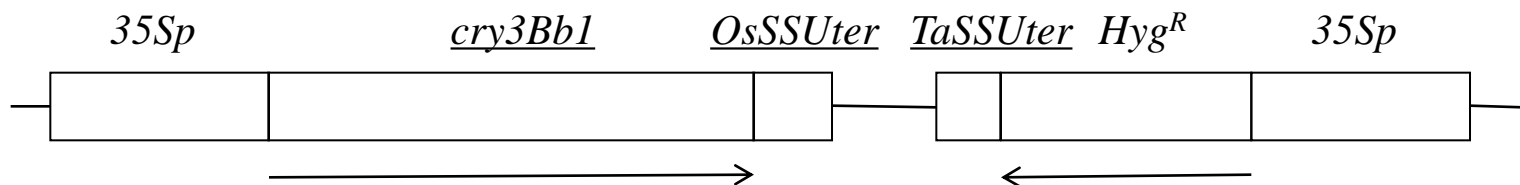
- Cry1Ab confers resistance to lepidopteran pests, such as the European corn borer (*Ostrinia nubilalis*) and species belonging to the genus *Sesamia*.
- Cry1Ab is a δ -endotoxin that selectively binds to receptors in the midgut of susceptible lepidopteran species
- The *cry1Ab* gene originates from *Bacillus thuringiensis* ssp. *kurstaki* and was introduced via *Agrobacterium*-mediated transformation.
- Southern blot analysis showed that only one copy of the gene construct was inserted



Event 2: Cry3Bb1

Trait and modification

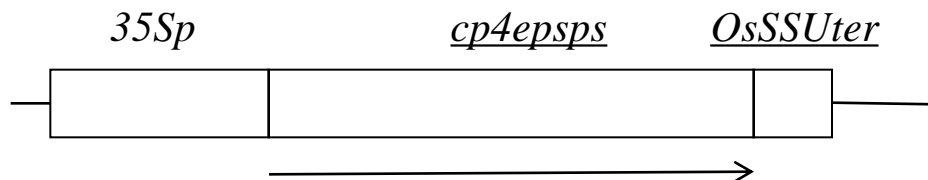
- Cry3Bb1 confers resistance to coleopteran pests, i.e. corn rootworms (*Diabrotica*) & Colorado potato beetle
- Cry3Bb1 is a δ -endotoxin that selectively binds to receptors in the midgut of susceptible chrysomelid species
- The *cry3Bb1* gene originates from *Bacillus thuringiensis* ssp. *kumamotoensis* and was introduced via *Agrobacterium*-mediated transformation.
- Southern blot analysis showed that only one copy of the gene construct was inserted



Event 3: CP4 EPSPS

Trait and modification

- CP4 EPSPS confers tolerance to glyphosate, the active ingredient of Round-up.
- The *cp4 epsps* gene produces a 5-enolpyruvyl-3-phosphoshikimic acid synthase which will take over the intrinsic plant EPSPS function (involved in aromatic acid biosynthesis) blocked by glyphosate.
- The *epsps* gene originates from *Agrobacterium* strain CP4 and was introduced via *Agrobacterium*-mediated transformation.
- Southern blot analysis showed that only one copy of the gene construct was inserted



GM triple-stacked event

Characterisation of proteins

- Cry1Ab, Cry3Bb1 and EPSPS do not show homology with toxic and allergenic proteins & are considered safe for human and animal consumption by Mexican authorities
 - EPSPS and Cry proteins have a different activity spectrum.
 - The Cry1Ab and Cry3Bb1 have a similar activity spectrum, but are active under different chemical conditions (midgut of Lepidoptera is alkaline (pH 10.5 -11), that of Coleoptera is neutral (pH 6.5-7))
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- The *cry1Ab*, *cry3Bb1* & *epsps* genes are expressed in all tissues (leaves, roots, inflorescences & seeds), **except in pollen**
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GM triple-stacked event

Phenotypic characteristics

- The GM triple-stacked line was similar to the corresponding single events and other commercialised lines regarding:
 - - vegetative vigour
 - - yield (seed production)
 - - flowering period
 - - pollen production & viability,
 - - seed dormancy
 - - resistance to target pests
 - - tolerance to glyphosate

Summary

- **Purpose:** cultivation of GM stacked event
- **Receiving environment:** Mexico
 - maize very important economically and culturally
 - landraces are grown
 - centre of origin of maize
 - sexually-compatible wild relatives (teosinte) are present
- **Traits:** resistance to pests (Lepidoptera & chrysomelids) & herbicide tolerance
 - Cry1Ab active against Lepidoptera
 - Cry3Bb1 active against Chrysomelidae
 - C4EPSPS confers tolerance to glyphosate
- **GM stacked event:**
 - phenotype & reproductive biology = non-GM maize
 - not shown to be more weedy
 - assessed as safe for human/animal consumption

Questions?



